NASA microgravity research highlights

Culturing a Future

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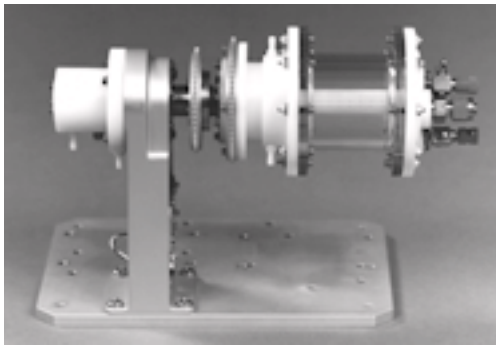
A New Tool

Researchers have been culturing cells for more than a hundred years. Despite this century's many technological advances, techniques for cell culturing have not changed significantly. Today, cells are typically grown in petri dishes or in flasks, just as they were a century ago. The cells are placed in these containers with a liquid media, a substance with nutrients the cells need to grow — and grow they do, in a flat layer on the bottom of the container. Cells grown in vitro, or outside the body, in two-dimensional layers do not behave in the same way as cells grown in vivo, or inside the body. In vivo cells grow three-dimensionally and form tissue that consists of cells that have changed their structure to perform a specific function in the body and other components, called matrix, that the specialized cells secrete. In vitro cells do not specialize, or differentiate. This poses obvious limits to research using in vitro cells to understand mechanisms that govern cell behavior and tissue formation, both normal and abnormal. It also puts the brakes on any attempts to grow tissue in vitro for replacement of defective or damaged tissue inside the body.



The tool that is revolutionizing tissue culturing: NASA's rotating bioreactor

Though the limitations of standard culturing practices have been apparent for some time, solutions have been slow in coming. In the 1970s, a small group at Johnson Space Center (JSC) began to think about space as a possible answer. The group theorized that if cells could be grown without the influence of Earth's gravity, they would not settle to the bottom of the culturing container; rather, they would be suspended in the media and therefore might assemble and form tissue that more closely resembles tissue in the body. Although the goal was to attempt tissue growth in microgravity, Pellis explains that the JSC group soon turned their efforts to creating a culturing device that simulated some aspects of microgravity on the ground: "If we had to take everything to space in order to conceptualize this device, it would be a long, arduous, and expensive process." The wiser path, and the one the JSC group took, was to first conceptualize the new culturing method on the ground.

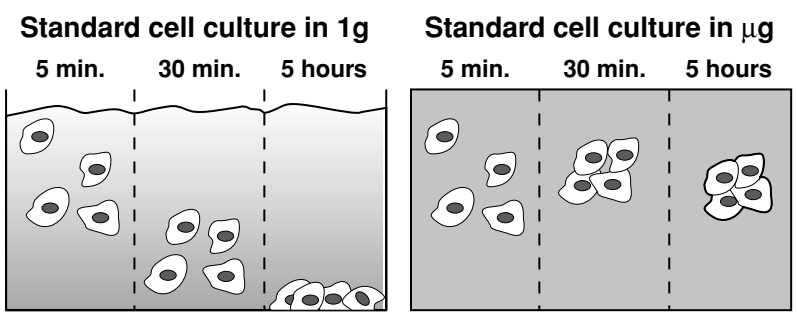
Stirring the cells in their containers to keep them from settling seemed a place to start. But stirring, as Pellis points out, can prove very deleterious to the cells: "Most cells do not like to get beaten around by mechanical shear or hydrodynamic shear." Hydrodynamic shear occurs when a liquid rolls over an object and wears it away. Like the action of water over rocks in a streambed, the stirred liquid media damages the cells as it rushes over and around them. The cells experience mechanical shear when they bump into the side of the vessel or are hit by one of the propellers used to stir the media. Pellis notes that there are primarily five cell types in the body that would be tolerant of the mechanical and hydro-dynamic shear created by stirring. Those include red and white blood cells and the cells that line blood vessels. "Those cells are equipped to handle that kind of damage, but everything else is really not happy in that environment," says Pellis. "The greater predominance of cell types that we would want to propagate are those that require a very quiescent environment. How do you keep cells suspended and yet have a very quiescent environment?"

The question consumed the JSC group through the mid-1980s. In 1987, three members of that group were close to a solution. David Wolf, then directing the effort to develop the new cell growth technology; Ray Schwartz, a bioengineer for Krug Life Sciences, Inc., a NASA contractor; and Tinh Trinh, an engineering technician also working for Krug, had as a team devised a system in which an upright cylindrical vessel — a bioreactor — about the size of a soup can was rotated using an electric drill. With this setup, they were attempting to establish what Pellis calls a "suspension modality." But the team had not been able to achieve this state with the model system. Then one day, Trinh decided to turn the rotating vessel on its side. That was the moment, says Pellis, that everything went into suspension: "That is how they discovered it. From there, they realized that as long as they kept the cylinder completely filled with fluid, the cells should remain suspended and no stirrer was needed." They called that first device the Slow-Turning Lateral Vessel (STLV), and it was ready for some serious testing.

An Early Believer

In 1987, J. Milburn Jessup was working at the University of Texas M. D. Anderson Cancer Center with his mentor, I. J. Fidler. Fidler's main interest was in understanding metastasis, or how carcinoma cells spread from a primary to a secondary site in the body. Fidler wondered whether there was something about the three-dimensional structure of a host tissue that made it susceptible to colonization by malignant cells. "We were thinking along the lines of trying to get some sort of culture system that would mimic some aspects of this three-dimensional growth," remembers Jessup.

Pellis, then also working at the Medical School of the University of Texas, Houston, and a friend of Jessup's, recommended that he go to JSC where an old colleague, Thomas Goodwin, was working with the group that was trying to devise a new culture system. The device Jessup saw when he went to JSC was far beyond the cylinder driven by an electric drill. Jessup remembers that when Schwartz and Wolf made the presentation of their system, he was impressed: "They had long-duration motors and parts that seemed to me to indicate that the engineering aspects of this were really very well thought-out. This bioreactor



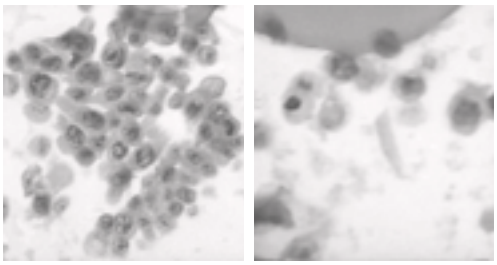
Cells cultured on Earth (left) typically settle quickly on the bottom of culture vessels due to gravity. In microgravity (right), cells remain suspended and aggregate to form three-dimensional tissue.

prototype was likely to be rugged and durable. At that point, they were looking for cells to put into it." Jessup was sure this system held promise. "I wasn't skeptical," Jessup recalls. "I was more drawn by how dedicated this group was to working hard to develop a product. They needed help in terms of resources and supplies, which we could offer, to make this reactor vessel functional. I didn't have any hesitation that this would in fact be a useful incubator. I thought it was perfectly logical."

Jessup's confidence turns out to have been well-placed. The JSC group had tried culturing hamster kidney cells in the STLV, but the results had not been what the group had hoped for. Jessup first provided JSC with a simple human colorectal carcinoma cell line. Then, when that met with success, the group wanted to attempt a cancer cell/normal cell interaction — a coculture. Jessup describes the result: "Fairly large tissue aggregates grew, and these had the ability to really recapitulate the morphology or appearance of what occurs in vivo in mice." Three-dimensional tissue masses, resembling a cancer tumor, had grown in the STLV. "Some years later," says Jessup, "those results were published. They demonstrated that the vessel was very good for these kinds of cocultures. We demonstrated a synergy not evident in other culture systems."

Work began on preparing a culturing system for a shuttle flight. Although the bioreactor would be in a microgravity environment aboard the space shuttle, and the cells would therefore be in suspension without vessel rotation, the same system developed for the ground was modified for use in space. The cylinder that is the rotating bioreactor is just a part of a larger system designed for keeping cells alive by providing all the resources they would have in a body. Pellis says that JSC had developed an integrated system: "It has a reactor — the culture vessel itself — but in addition, it has its own 'lung,' its own 'heart,' its own food supply, and its own waste management." Adapting that system for space hardware rather than starting from scratch made the most sense.

Jessup was the first investigator to use the space hardware, although he explains that his experiment was primarily a test of the space system. JSC designed the experiment, and he provided the cells and the analysis. Once again, Jessup was providing resources that JSC needed in its quest to make their rotating wall bioreactor a functional, useful device. "The hardware was really developed before decisions were made as to the development of experiments that would go in it," says Jessup. "At least for this type of research, that is not necessarily bad, because in cell biological research, everyone uses the same types of culture tools. People in cell biology all use a standard set of petri dishes or flasks for everything they do, so the vessel, once you build it, can be used for multiple purposes."



These photos compare the results of Jessup's 1995 flight and ground control experiments in colon carcinoma culturing. The cells grown in microgravity (left) have aggregated to form masses that are larger and more similar to tissue found in the body than the cells cultured on the ground (right).

After several spaceflight trials, JSC's Bioreactor Demonstration System flew onboard the shuttle in July 1995 as part of STS-70, with cells provided by Jessup. The experiment was not only an engineering success but also a scientific one. Jessup's sample of colon carcinoma cells aggregated to form masses 10 mm in diameter. These masses were 30 times the volume of those grown in the control experiment on the ground. The experiment was repeated in August 1997 on STS-85, and mature differentiated tissue samples were grown again, confirming the previous results — microgravity was an environment beneficial to cell culture and tissue growth.

A Life of Its Own

Although Jessup was an early believer in NASA's rotating bioreactor, Pellis recalls that others had doubts. "The biological scientific community was rightfully skeptical of something that was such a radical change," says Pellis. In the early 1990s, a small group of about a dozen interested investigators from universities were coming to JSC to try the rotating bioreactor in ground-based studies. As documentation of their results started to appear in established journals, word slowly began to spread. The word, according to Pellis, was that "you could get three-dimensional tissue arrays that looked like more than just globs of cells." The three-dimensional arrays had specific characteristics that made them identifiable as a particular kind of tissue. "For instance," explains Pellis, "when you placed a cartilage sample in the system and propagated it, the piece of tissue that grew, when you cut it and analyzed it like a pathologist, looked like a piece of cartilage. The same was true of colon cancer, prostate cancer, and lymphoid tissue."

As interest grew in this new culturing system, supply rose to meet the demand. In the early 1990s, while investigations were being conducted at JSC, Ray Schwartz and another Krug employee formed a new company, Synthecon, Inc., to manufacture the rotating bioreactor. NASA licensed them to produce the system, and with the help of some short-term Small Business Innovation Research awards from NASA, Synthecon had the systems, which they called rotating wall vessels, available on the commercial market by 1994. Commercial availability has meant that many groups that have no connection to NASA now have the rotating bioreactor. Pellis interprets the use of the bioreactor by private companies as a healthy sign: "I don't think NASA is going to know everything that everyone is doing with these things. It means that now the interest is running on its own inertia."

Pellis has his hands full tracking and supporting the research of scientists who are working with the rotating wall vessel under NASA grants. When Pellis took over as director of NASA's tissue culturing program in 1994, there were 14 investigators formally sponsored by the program. Today there are approximately 125 investigators using this technological approach in their research. That growth is not only the natural result of the spreading of the word through the discipline literature, but also the result of JSC taking the word out to the scientists. Pellis reports that the JSC group has put on workshops and symposia at the meetings of the major cell science societies, including the Society for In Vitro Biology, the American Society for Microbiology, and the American Society for Cell Biology, and met with an enormous amount of interest in the technology. Responses to the NASA Research Announcement in biotechnology have increased accordingly, and Pellis notes that interest in the rotating bioreactor is developing, for the most part, on two sides of a fence: "One side is applications, meaning building tissue, whether it is tissue for research or for transplantation. The other side is study of those properties of cells that change because the cells are in freefall."

Building Tissue

Pellis believes that in the next five to ten years, the rotating bioreactor will begin to routinely produce tissue for research and transplantation. The tissue produced to date in the rotating wall vessel has already offered unique research opportunities. "This is the first time," says Pellis, "that we have a look at the dynamics of a three-dimensional arrangement in a cultured setting. For instance, we can grow a human colonic polyp from individual cells. Observing that particular three-dimensional dynamic is an investigation of cancer that can lead to the development of therapeutic treatments. That is not something from 'Ripley's Believe It or Not.' That is going to happen."

The National Institutes of Health (NIH) also believe it is going to happen. Sixteen research projects involving tissue culturing in

the rotating bioreactor are currently under way at the joint NASA/NIH Center for Three-Dimensional Tissue Culture at the Institute of Child Health and Human Development. The two agencies joined to form the center in 1994 under an agreement that the NIH would provide the lab and NASA would provide rotating bioreactors and other support. The combination of NASA technology and NIH expertise has already resulted in the successful culture of several infectious agents that are difficult to grow and control in a culture setting. Pellis points to the growth of Cyclospora, a parasite that lives in berries and causes extreme gastrointestinal distress when eaten, as an example of the project's success: "No one has been able to grow Cyclospora in culture until this year, when researchers at the joint center took a new approach and cultured the organism with cells from the small bowel." The tissue samples grown in the rotating bioreactor at the center are being used to design therapeutic drugs or antibodies, "or alternatively," says Pellis, "for designing a strain of the organism from which a vaccine could be produced." Cyclospora is not a big threat in America, but worldwide it is responsible for a significant percentage of infant deaths from dehydration. Researchers at the joint center have also had success culturing the human immunodeficiency virus (HIV-1). Pellis acknowledges that HIV has been propagated before without the rotating bioreactor, but at the joint center, the NASA technology has made possible the propagation of the virus in human lymphoid tissue. Those samples are giving scientists an opportunity to observe the virus in full dynamic process, which should provide a new perspective on the disease and on possible treatments.

Tissue engineering for transplantation is also progressing well with several projects that are, according to Pellis, "close to fruition." Closest of these is a project to culture human pancreatic islet cells for transplantation into diabetic patients for the control of insulin production. A company called VivoRx is currently using the rotating bioreactor to culture the differentiated pancreatic cells, which are then encapsulated in treated seaweed membranes to make them acceptable to the human immune system. Once transplanted, the cells secrete the appropriate amount of insulin for regulating the body's blood sugar levels. In the rotating bioreactor, the small number of pancreatic cells provided by donors will be expanded to the number of cells required to successfully treat patients presently requiring daily insulin injections. The encapsulated cells are currently being tested in human patients. Jessup finds this use of the rotating bioreactor particularly impressive: "There really is a useful market for the bioreactor in that type of application. Anyone working on the transplantation of organs may be able to use the bioreactor's properties to expand or cultivate cells."

At Kennedy Space Center, Timothy Hammond (left) and James Kaysen (right), of the Tulane Environmental Astrobiology Center, and Thomas Goodwin (center), of JSC, ready their cell experiment for its April 1998 shuttle flight in the Bioreactor Demonstration System (shown on table).

A Novel Look

The other side of the fence in bioreactor research is using the culture technique to gain what Pellis calls "a novel look at the cell." Pellis notes that while using the bioreactor to engineer better tissue samples, several researchers have observed that cells adopt some interesting adaptive modes while freefalling in the rotating bioreactor on the ground and in orbit. Pellis believes that by watching the reaction of the cells to the new environment of suspension or microgravity, scientists will discover more about the mechanisms that control the cells' behavior. "Besides being able to grow tissues," says Pellis, "we now have a new and fascinating way to see inside the cell."

Timothy Hammond is an example of a researcher who started out using the bioreactor to engineer tissue and ended up using it to find the mechanisms within the cells that control differentiation. Hammond and his team at the Tulane Environmental Astrobiology Center, which is jointly sponsored by Tulane University, Xavier University, and the VA Medical Center in New Orleans, were studying protein receptors that bind common toxins in the proximal tubule, a microscopic tube in the kidney. Hammond explains that the kidney often is damaged by drugs and toxins contained in strong antibiotics. "We were interested in the proteins that get bound in the kidney by these toxins. We wanted to culture the cells that make these proteins, to develop protective agents," says Hammond. "But the problem is that there is no cell culture line that expresses the relevant proteins. If you take a kidney cell and put it into a cell culture, a day later it no longer has any of its special features. So we had a lot of interest in finding a cell culture method that would keep the special features of tissues intact."

Hammond tried over 400 different cell types and cell culture techniques searching for a way to retain the special features of the differentiated cells of the tubule, such as microvilli, hair-like structures found in some tissues. He met with no success. Then he read about results of culture experiments in the rotating bioreactor, and he immediately contacted NASA. "We tried the rotating wall vessel," remembers Hammond, "and to our shock, surprise, and delight, the tissue was beautiful. All the hair, the microvilli, grows on the cells, and they express all the specialized proteins we needed. The results were very dramatic."

Though these results were striking, Hammond thought that culturing the cells in space might produce even more spectacular samples because in orbit, the tissue masses would not be limited by size. On Earth, when the cells aggregate into three-dimensional masses in the rotating bioreactor, they eventually reach a size at which they are too heavy to be suspended by the rotating action of the vessel. If the rotation is increased to keep the aggregates suspended, they are thrown against the vessel wall, which damages the tissue. "If we were truly going to understand how different cells grow together to form a tissue with all its medical implications," says Hammond, "we had to find some way to get out of the limits caused by gravity. That is why we wanted to fly the renal tubular cells."

Hammond's first opportunity to conduct an experiment in microgravity was during the sixth Mir research increment, from September 1997 to January 1998. Hammond chose to grow rat renal tubular cells in NASA's Biotechnology Specimen Temperature Controller, a cell incubator, onboard the Russian space station, Mir. He chose rat kidney cells for his sample because he needed cells that would grow and differen-tiate over the entire four

During the STS-90 shuttle flight in April 1998, Hammond's human renal tubular cells formed large tissue aggregates (visible as white masses in the lower left corner of the sample above). Hammond was able to use the samples to identify key genes in the control of differentiation.

Astronaut David Wolf makes notes about Hammond's sample of rat renal tubular cells (above his head) onboard Russian Space Station *Mir*. Wolf is one of the co-inventors of NASA's rotating bioreactor.

months to help verify the function of the hardware. Hammond reports that on Mir, the tissue aggregates "grew beautifully" under the care of astronaut David Wolf, one of the rotating wall bioreactor's three inventors. "We got gorgeous cell aggregates, bigger than the aggregates grown in the control experiment on the ground," says Hammond. "And we saw the proteins that we were interested in, the tubular toxin protein receptors, expressed in flight."

Hammond was pleased with these results, but he wanted to know what mechanism in the cells was causing differentiation and expression of the desired proteins in microgravity. Genes control these functions, but identifying which genes are doing the controlling out of the millions present in a cell is close to impossible. Hammond reasoned that a comparison of the genes that are active in the cells during culturing in spaceflight to those that are active in culture on the ground might help in pinpointing the specific genes responsible for differentiation. In early 1998, Hammond cultured a sample of human renal tubular cells in the Bioreactor Demonstration System on the space shuttle for six days. Hammond reports that by comparing the activity of 10,000 genes in the flight and ground cultures, several of the control genes for differentiation and three-dimensional tissue formation were identified. Hammond eventually wants to use these findings to make kidney implants for hormonal therapy. "With the knowledge of the control genes," says Hammond, "we could control the proteins produced by tissue in the rotating vessel by genetic manipulation so we can give the patient a better, longer-lasting implant. Our experiment is a very exciting piece of basic science, but it does have clinical correlatives." Hammond is certain that the rotating wall vessel will bring such success to many other researchers in the future. "I believe that NASA's biotechnology program is going to revolutionize the whole field of cell biology," says Hammond. "In fact, it already has."

A Keyhole

While the rotating bioreactor is providing researchers with a new way to see inside a cell, it is also expected to contribute to our efforts to look out into our solar system and beyond. Jessup and Pellis share the view that research conducted in the rotating bioreactor will be a prerequisite for space travel and colonization. Jessup, currently at the University of Pittsburgh Medical Center, serves as the chairman of the microgravity biotechnology discipline working group, which advises NASA about the potential and direction of the research program. Jessup sees the primary role of the cell culturing program as helping to ensure astronaut health. The program can do this, he says, through research that "provides the underpinnings for many of the health disorders that occur in space, such as anemia, bone matrix loss, and kidney stone formation." Jessup points to investigators already using the bioreactor to solve these problems. Among them are Pellis, who has done work examining the behavior of immune cells in microgravity that may lend insight into the changes astronauts experience in their immune systems during spaceflight, and Lisa Freed and Gordana Vunjak-Novakovic, researchers at the Massachusetts Institute of Technology, who believe that results from their experiment to grow cartilage on Mir might provide clues for understanding why astronauts experience a weakening of muscle and bone while in space. (See the profile of Freed and Vunjak-Novakovic for more about their experiment.)

Jessup also sees a continued role for the rotating bioreactor once astronauts are en route to new planetary destinations. The bioreactor can provide a means for culturing red blood cells or skin in the event of astronaut trauma. It can also be used to culture unicellular organisms like blue-green algae as a supplemental food source or a means of replenishing the air supply for the spacecraft or for a planetary colony. "Because such organisms are biologically renewable," Jessup says, "they may be cheaper in the long run than chemical agents that could be used to create air and easier to transport." Pellis adds that there is potential for using the findings from bioreactor research to send cells into space as exploratory probes. Cell cultures could be designed to respond to environmental conditions of other planetary bodies in such a way that scientists could judge whether an environment is suitable for life. "Using these probes," says Pellis, "we could determine if the atmosphere is supportive of cells, if there is water, or if the environment is amenable to propagation."

Though Jessup is enthusiastic about the contributions the bioreactor will make toward engineering tissue on Earth and toward the study of novel aspects of cell biology, it is the program's role in future space exploration that he finds most compelling: "In the next millennium, we will move off the Earth," says Jessup, "and quite frankly, I think that this bioreactor technology is the primitive forerunner of the technology that will enable us to do that. The bioreactor represents a keyhole to the future.

Pellis also believes that the bioreactor is the keyhole to a "new era" in tissue culturing for research and applications both on Earth and in space. That belief is why he accepted NASA's offer in 1994 to become director of the microgravity tissue culturing program. Says Pellis, "The opportunity for discovery in this field is high, and I am one researcher that was baited and hooked by it. I think that for some cellular functions, we are going to see a real step up in understanding. I did not want to be just someone in the stands watching this happen."

Additional information

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